

Original Research Article

Echinacea purpurea* promotes maturation of dendritic cells *in vitroMuftah El-Feituri^{2,3}, Diethelm Wallwiener², Brigitte Gückel², Simone Kayser², Hinnak Northoff²²Department of Transfusion Medicine, University of Tübingen, Germany³Faculty of Public Health, Al-Arab Medical University, Germany***Corresponding author**

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Abstract: In this study, we analysed the effect of *Echinacea purpurea* on the maturation of monocyte derived dendritic cells (mDC) of healthy human donors. *Echinacea p.* was either used as a single maturation stimulus or in addition to various concentrations of IL-1 β , TNF- α , and IL-6. Used as a single stimulus, *Echinacea p.* induced an upregulation of DC markers such as CD83 and CD80 as well as the expression of the chemokine receptor CCR7. Furthermore, in combination with IL-1 β , TNF- α , and IL-6 *Echinacea p.* showed a clear additive effect on DC maturation. This was accompanied by an increase in PGE2 in supernatants of mDC cultures. Our study implies that *Echinacea p.* is able to trigger mDC to enhance their antigen presenting functions and migration capacity which could result in a more efficient activation of resting T-cells in the lymphoid compartment.

Keywords: *Echinacea Purpurea*, dendritic cells, proinflammatory cytokines, prostaglandin E2, chemokine receptor 7, antigen presentation.

INTRODUCTION

Echinacea purpurea L., Asteraceae, is a widely used herbal medicine to treat respiratory tract infections based on its immunostimulating properties. Although several clinical studies claim health benefits in the therapy of common cold its effectiveness has not been proven beyond a reasonable doubt [1-3]. However, pharmacological effects of *Echinacea p.* on effector cells of the immune system – in particular activation of macrophages and natural killer cells - have been demonstrated convincingly [4-7]. Several studies have shown that upon contact with *Echinacea p.* macrophages release proinflammatory cytokines [5,8].

DC play a key role in the initiation of adaptive immune responses. Depending on their maturation stage they can prime immune responses or have the ability to induce tolerance [9]. While immature DC are efficient in capturing and processing antigens, only mature DC are able to prime naïve T-cells after antigen presentation. DC maturation can be induced by so called “danger signals” such as bacterial and viral products, heat shock proteins, as well as by proinflammatory cytokines. Mature DC are mainly characterised by the expression of CD83 and an upregulation of several costimulatory molecules [10,11]. Together with PGE2 the chemokine receptor CCR7 is necessary for DC migration from the sites of infection to secondary lymphoid organs guided by its

ligands CCL19 and CCL21 [12,13]. Recently it was shown that CCL19 and CCL21 further have an anti-apoptotic effect on DC via binding to CCR7 [14]. In our study, we analysed the influence of *Echinacea p.* on immature DC with regard to activating effects on this cell population. In this context we focused our investigation on the expression of “maturing molecules” like CD83 and CCR7 as well as on the secretion of PGE2.

MATERIAL AND METHODS***Echinacea purpurea***

The *Echinacea purpurea* preparation used was a pressed juice from *Echinacea p.* (1.7 - 2.5:1, kindly provided by MADAUS AG, Cologne, Germany), conserved with ethanol (18%), freeze-dried and reconstituted in endotoxin-free X-Vivo-medium (Biowhittaker, Verviers, Belgium) to make final concentrations as indicated.

The endotoxin concentration, as determined by *Limulus* assay (Pyrochrom; Haemochrom, Essen, Germany) was <21pg endotoxin / mg *Echinacea p.*

Dendritic cells

mDC were cultured in X-Vivo-15 medium supplemented with 2 mM L-Glutamine (Life Technologies, Paisley, UK), 100 μ g/ml Streptomycin, 100 U/ml Penicillin (Biochrom, Berlin, Germany) and

1% of autologous plasma or pooled AB-serum (ccPro, Neustadt, Germany) ("mDC-medium"). Immature mDC were generated from peripheral blood mononuclear cells (PBMC) of healthy donors enriched by plastic-adherence and cultured in mDC-medium supplemented with 1000 U/ml IL-4 (R&D-Systems, Wiesbaden, Germany) and 100 ng/ml GM-CSF (Immunex, Seattle, USA). At day 7 nonadherent immature mDC were collected and cultured for additional 48 h in mDC-medium containing IL-4 and GM-CSF further supplemented with or without a standard cytokine-cocktail ("cyt.-cocktail") consisting of IL-1 β (8 ng/ml) (R&D-Systems), TNF- α (8 ng/ml) (PAN Biotech, Aidenbach, Germany) and IL-6 (8 ng/ml) (R&D-Systems) or 1:20 dilutions of the standard cytokine-cocktail. Where indicated, *Echinacea* (1.8 mg/ml) or PGE2 (833 ng/ml) (Pharmacia & Upjohn, Erlangen, Germany) was added.

Flow cytometry

Phenotypisation of nonadherent mDC was performed using the following monoclonal antibodies by fluorescence activated cell sorting (FACS): IgG₁-FITC, IgG_{2a}-FITC, IgG₁-PE, IgG_{2a}-PE as isotype controls, CD14-FITC, CD80-PE (all from BD Bioscience, Heidelberg, Germany,) CD86-FITC, CD54-FITC (both from Diaclone, Besancon, France), CD83-PE, (Coulter Immunotech, Marseille, France), CCR7-PE (R&D-Systems). Cells were analyzed using a FACS Calibur and Cell Quest software from BD Biosystems.

PGE2-ELISA

Secretion of PGE2 was determined by ELISA in culture supernatants (SN) of mDC according to manufacturer's instructions (R&D-Systems).

RESULTS AND DISCUSSION

Immature mDC of five healthy donors were cultured with or without the addition of 1.8 mg/ml *Echinacea p.* This concentration has been demonstrated before to enhance pathogen-stimulus dependent cytokine production in leucocytes (own unpublished data). FACS staining revealed an increase of a CD83+ and a CD80/86+ cell population in *Echinacea p.* treated mDC cultures compared to non stimulated control mDC (Fig. 1). Interestingly, *Echinacea p.* also induced the expression of the anti-apoptotic and migratory marker CCR7. These results indicate the differentiation of a distinct mature mDC fraction by using *Echinacea p.* as a single activating stimulus.

To investigate additive effects on cytokine-matured DC, *Echinacea p.* was added to immature DC from four different donors together with the cytokines IL-1 β , IL-6, and TNF- α . Addition of *Echinacea p.* provoked a more pronounced upregulation of CCR7⁺ cells in all cases tested. CD83 and CD80/86 expressions could be enhanced by *Echinacea p.* in mDC of donors 1

and 2. In these cultures DC maturation with cytokines alone was not as complete as achieved for donors 3 and 4 (Figure 2A). The most dominant effect of *Echinacea p.* on CD83 and CD80/86 expression was observed for donor 2, who showed a suboptimal mDC maturation using the standard cytokine-cocktail alone. As PGE2 is a key factor for CCR7 expression of mDC matured with proinflammatory cytokines, its exogenous addition is necessary to obtain functional mDC *in vitro* [15]. We asked, whether the effects of *Echinacea p.* treatment could mimic PGE2-induced CCR7 expression. Our data demonstrate that using *Echinacea p.* for DC maturation resulted in a similar proportion of CCR7⁺ cells as compared to PGE2 treatment (Figure 2B).

Recent studies showed that besides CCR7 expression, PGE2 itself is necessary to control DC migration by facilitating CCR7 dependent signal transduction [16,17]. We quantified PGE2 secretion in mDC and found a significant upregulation of PGE2 in the presence of *Echinacea p.* in cytokine-cocktail matured mDC (Figure 2A). These PGE2 concentrations (2-6 ng/ml) appear to be low, however, Scandella *et al.* [17] demonstrated that concentrations about 15 ng/ml PGE2 can significantly enhance CCL19- and CCL21-mediated migration of mDC. Zeller-Rieser *et al.* [15] demonstrated that the PGE2-synthesis of mDC is suppressed by IL-4 *in vitro*. Indeed, in the presence of IL-4, which is always present in our DC-cultures we found no PGE2-secretion - neither in immature mDC nor after maturation by the cytokine-cocktail - however addition of *Echinacea* resulted in PGE2 induction in non-cytokine-cocktail as well as in cytokine-cocktail matured DC (data not shown and Figure. 2A). In accordance with this observation, IL-12p40 secretion - known to be induced by PGE2 - [18] was amplified up to 3-fold in the presence of *Echinacea p.* (data not shown).

We further asked whether *Echinacea p.* could overcome suboptimal DC maturation due to lower cytokine concentrations. In our study suboptimal DC culture conditions (1:20 dilution of standard cytokine concentrations) resulted in an upregulation of CD83 and CD80/86 if *Echinacea p.* was added. This effect was more pronounced than the additive effect under standard cytokine conditions. Furthermore the addition of *Echinacea p.* to DC matured with diluted cytokines induced a CCR7 expression as high as under standard cytokine-concentrations (Figure 2C).

As DC play a master role in the initiation of adaptive immune responses, we were able to propose a way in which *Echinacea p.* could link innate and adaptive immune responses. Considering health benefits on respiratory infections it can be assumed that *Echinacea p.* enforces antigen presentation by enhancing DC maturation and migration. *Echinacea*

contains numerous chemical constituents. Although arabinogalactan was demonstrated to induce IL-1 β and TNF- α -secretion in macrophages [4], there is still no consensus on exactly which component is the most active immunomodulator as well as on the optimal route of administration (oral vs. parenteral). Future studies should tend to detailed analysis of the single

components of *Echinacea p.* as well as to functional characterisations of Echinacea-matured DC *in vitro* and *in vivo*. Last but not least as *Echinacea p.* is a safe and well-tolerated compound, [19,20] it seems worth to verify its potential as an adjuvant for dendritic cell based immunotherapies.

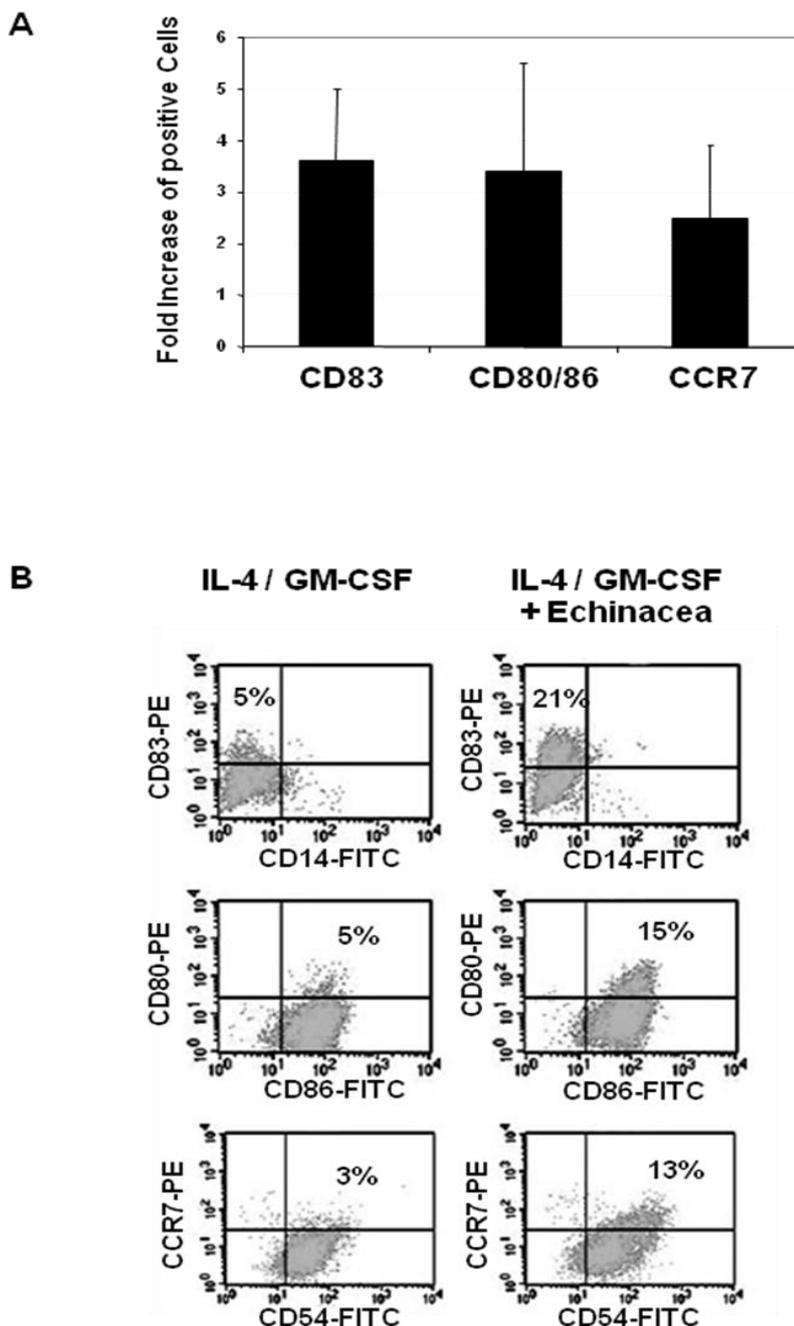


Figure 1. Effect of Echinacea on immature DC. Immature dendritic cells were generated as described in materials and methods using autologous plasma. On day 7 IL-4 and GM-CSF was substituted and cells were cultured in presence or absence of Echinacea. On day 9 mDC were harvested and compared by FACS-analysis. (a) Expression of analysed markers are shown as fold increase of positive cell fractions following Echinacea-treatment relative to non Echinacea-stimulated control mDC (normalised to 1). The values represent the average of four (CD83) or five (CD80/86, CCR7) different donors, respectively, +/- SD. (b) DC phenotypisation by FACS of one representative donor shown as two colour-stainings in dot-blots.

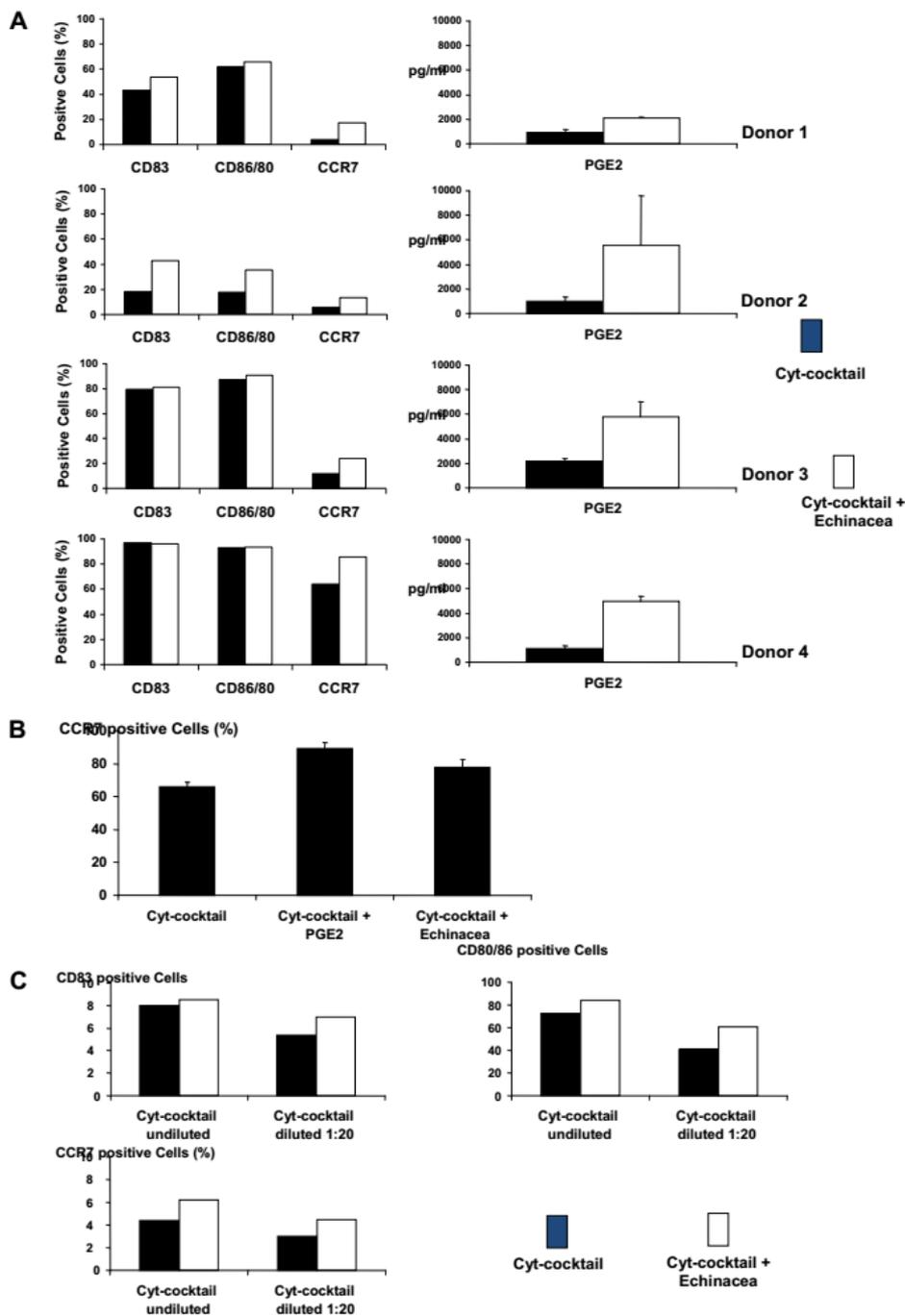


Figure 2. Effect of Echinacea on cytokine-mediated DC maturation. Immature DC were matured in mDC-medium containing pooled AB-serum for 48 h using IL-1 β , IL-6, and TNF- α (cyt. cocktail). IL-4 and GM-CSF was substituted. DC cultures were further supplemented or not with Echinacea or PGE2, respectively. Differently generated DC-subsets were analysed for the expression of CD83, CD80/86 and CCR7 by FACS-analysis. (a) Immature DC of 4 different donors (D1-D4) were either matured using the standard cyt.-cocktail alone (black bars) or in presence of Echinacea (open bars). Expression data were determined by FACS-staining. Supernatants of DC cultures were used to analyse PGE2 secretion. PGE2 concentrations were measured using a standard ELISA in duplicates (+/- SD). (b) CCR7 expression of mDC stimulated with the cyt.-cocktail with or without further addition of PGE2 or Echinacea. Results represent means of two different donors (+/- SD). (c) Results of CD83, CD80/86 and CCR7 expression of mDC stimulated with the cyt.-cocktail undiluted or 1:20 diluted with (open bars) or without (black bars) addition of Echinacea IL-4 and GM-CSF was added in standard concentrations.

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